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Studies on the Overwintering and Modes of Infection of the Fire Blight Organism

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Studies on the Over-wintering and Modes of Infection of the Fire Blight Organism[1,2]

E. C. Tullis

Economic Importance of Fire Blight in Michigan

Fire blight in Michigan ranks among the more important of the diseases of the apple and pear and periodically causes heavy losses to the grower. These losses are brought about mainly through the killing of fruiting wood. In severe cases, several years will elapse before the tree replaces the lost parts with new growth and reaches the stage of production possessed before it became diseased.

The development of the pear industry in this state has largely been limited by this disease. Certain regions in the eastern and southern part of the state have been forced to abandon this crop chiefly because of the losses caused by blight. The remains of many of these old orchards may still be seen in various parts of the state.

The apple as a rule has not suffered as severely from the disease as has the pear, but, in the past decade, many apple orchards have sustained very severe losses. Certain observations, which have come under the writer's attention, may be cited as examples.

Records taken at the Huron Farms Company orchard during the summer of 1926 showed that of a block of 1,508 apple trees, 166 had been so badly damaged by blight, during a period of three years that they had to be removed. Root and crown injury in this same block was so severe that a number of trees had to be bridge-grafted. Sixty-one trees had been bridge-grafted in the spring of 1925. These trees were girdled and approach grafts were set in 1926 because it was found that this treatment was more satisfactory than attempting to clean up the cankers below the ground line and make bridge grafts to the roots. One hundred fifty-nine additional trees were girdled and bridge grafted in the spring of 1926.

Losses in one block of 323 pears in this same orchard amounted to about 20 per cent of the trees. These trees were so badly blighted that, though every attempt was made to save them, they were lost because so little was left after the diseased limbs had been removed.

A small pear orchard of 30 trees was observed during the summer of 1927, on the west side of the State, south of Saugatuck. Fully 90 per cent of the trees were blighted. Twenty-five of these trees were removed the following spring.

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The writer wishes to express his thanks to Dr. C. W. Bennett for valuable suggestions given during the progress of the work and also to Dr. E. A. Bessey and Dr. G. H. Coons for criticism of the manuscript; to F. C. Bradford and H. A. Cardinell for materials used and suggestions.

While the orchards described above represent some of the cases of severe attack which have been observed during the past three years, they are by no means unusual or isolated examples of the ravages of blight. The disease is present and causes throughout the state a certain amount of damage in a considerable number of orchards every year.

The data in Table 1 obtained from the Plant Disease Reporter, for the years 1921-1925 inclusive, represent a comparison of the estimated percentage losses to the apple crop in Michigan, from fire blight and other diseases. The table shows that losses due to fire blight, as estimated by the collaborators of the Plant Disease Survey, U. S. Department of Agriculture, have been for this period approximately 1 per cent lower than those due to scab, commonly recognized as the most serious apple disease.

Table 1
Percentage losses of the apple crop due to fire blight and other diseases

Year	Fire blight	Scab	All other diseases	Total
1921	5	2	1	8
1922	0	16	0	16
1923	5	1	1	7
1924	5	12	2	19
1925	8	2	3	13
Average	4.6	6.6	1.4	12.6

History of the Disease

Fire blight has been known for about 150 years. The disease apparently is indigenous to North America and no doubt occurred on wild hosts previous to the introduction of the apple and pear into this country. Among the wild hosts listed in North America are wild crab, hawthorn, service berry, the red berried California holly, mountain ash, and the loquat. The disease has also been reported from Japan, New Zealand, and Italy. It was first described in 1794 but had been observed almost 15 years earlier by William Denning in the Hudson River Highlands. The disease spread westward and southward into the Mississippi valley and it was not until 1900 that it had reached California and in 1915 it had become a serious disease in central Washington.

Nothing was known as to the cause of the disease for almost one hundred years after the disease was described. Various explanations were made in an attempt to account for its appearance. Among the causes listed were electricity, sunscald, frozen-sap, old age, over-nourishment, under-nourishment, fungi, insects, and other factors.

Review of Literature

In 1878, T. J. Burrill (5), then botanist at the University of Illinois, advanced the suggestion that fire blight was due to a bacterial infection. This revolutionary idea was substantiated by his experiments which he reported in 1881 and by the work of Arthur (1) in 1885. Waite's (28) contribution in 1894, demonstrating the origin of blossom infection through the agency of bees, wasps, and other nectar-seeking insects, has been fol-

lowed and supplemented mainly by the work of Gossard and Walton (12). Investigations by Jones (16), Stewart and Leonard (27), Merrill (18) and A. C. Burrill (4) have established the relation of various species of chewing and biting insects to twig infection. Jones (16), Lathrop (17), and A. C. Burrill (4) have also made contributions showing that aphids may similarly transfer bacteria to growing twigs. Contributions to our knowledge of the over-wintering of the organism have been made by Sackett (23), Whipple (29), Brooks (6) and Nixon (19). Miss Bachmann (2) and Nixon (19) have shown the manner of intercellular migration of the organism in the invasion of the host. The importance of wind-blown rain has been demonstrated by Stevens and his coworkers (25), Gossard and Walton (12), and others. Heald (15) first recognized the fact that leaf invasion may produce a leaf blight.

Scope of the Investigations

The investigations reported in this paper were begun in the fall of 1925 and have continued through the summer of 1928. The writer spent five months during the summer of 1926 and the greater part of the summer of 1927 in the Huron Farms orchards near Ann Arbor. Trips were also taken into various parts of the southern half of the State to examine blighted orchards. The remainder of the work was done at the Michigan State College, Department of Botany.

The problems under consideration in this paper relate to overwintering of the causal organism, *Bacillus amylovorus* (Burr.) Trev., to dissemination of the disease by aphids and by meteoric water, to certain problems of infection, and to the production of twig blight.

Over-wintering

It is a well established fact that the fire blight bacterium over-winters in the cankers produced on the limbs and trunks of apple, pear, quince and other host plants. The percentage of cankers in which the causal organism remains alive through the winter in Michigan orchards has been found to vary considerably in different seasons and under different conditions of vigor of the trees. Time of infection and weather conditions during the late summer and fall seem to exert an important influence at times. It has been commonly reported, and experimental evidence seems to show, that the region of over-wintering is along the edge of the canker or in apparently healthy tissue just in advance of the canker edge.

Whipple (29) reported that the pear is probably the most important host for the over-wintering of the fire blight bacterium in Colorado, and Sackett (23) succeeded in recovering the organism from 21 of 83 blighted pear twigs. On the other hand, Reinking was unable to isolate the organism from apple during the fall, winter, or spring following infection in Wisconsin. Brooks (6) found that over-wintering occurs in the apple in twigs as small as three-tenths inch in diameter. He also reported that the organism was isolated as far as one inch in advance of the canker edge in dormant cankers.

Nixon (19) has observed that pockets are formed in the tissues of the bark along the margin of the canker. These pockets are filled with masses of a jelly-like substance in which the organisms are embedded. He considers that these masses or "cysts" represent the organism in its over-wintering

condition. The work of different investigators seems to show that these "cysts" may not be necessary. These so-called "cysts" have in the past been referred to by most investigators as "pockets" and were supposed to be the seats of over-wintering.

Some cankers were observed by the writer in which the spring exudate came first from the more central part of the diseased area of the bark. These observations led to an attempt to determine whether the organism may not in some cases remain alive in bark which is either dead or contains many dead cells.

Two types of cankers have been observed on both apple and pear. The first may be called the determinate type of canker in which a definite margin is very evident. This is caused by the formation by the host of a layer of cork in advance of the canker edge. The second may be called the indeterminate type, because cankers which fall into this category have no definite edge and the determination of the extent of the affected area is possible only by cutting through the outer bark. Cankers which have not been checked by drought or other factors are most commonly of the second type. These may result from infections of the late summer or fall. Cankers formed as a result of spring and early summer infections usually are of the determinate type. Some of these cankers seem to harbor live bacteria during the greater part of the summer and fall season, and, in some, the bacteria seem to renew activity in the fall after a period of slow development during the summer.

During the fall of 1926 in making incisions into a number of pear and apple cankers of the indeterminate type, it was found that there were certain areas outside the cambium layer in such cankers, which had a rather dark and water-soaked appearance. Closer observation indicated that these areas contained bacteria in enormous numbers. It was thought worth while to mark a number of cankers of the type described above and make isolations at regular intervals throughout the winter and spring to watch for earlier signs of bacterial activity in the spring.

Accordingly, during the winter and spring of 1926 and 1927, observations were made on the over-wintering of the blight organism in pear and apple cankers. Isolations were made under the microscope by a modified capillary tube method, which served very satisfactorily to obtain cultures of the organism. In only a few cases did contamination result. Bits of tissue, removed aseptically, were mounted in sterile water on a slide. Capillary tubes were drawn from 2 mm. soft glass tubing and fastened in a modified Barber manipulator, and the capillary tube was then maneuvered until the tip was observed to be over a mass of the organisms as they exuded from the tissue. The tube was then lowered into the suspension of the organism and quickly withdrawn. The portion of the tube containing the bacteria was then broken off by means of sterile forceps and dropped into a tube of bouillon. Clouding usually occurred within 48 hours. These cultures were then inoculated into young apple twigs by puncture. The plants were incubated in the chambers as shown in Plate I, figure C.

Isolations were made during the months of January, February, March, and April. The first exudate in the spring of 1927 was observed April 30 on pear at Ann Arbor. Cankers of apple and pear were used in making the isolations and are so designed in Table 2. On March 8, three cultures were obtained from the dead tissue 12 inches back of the canker edge from an indeterminate type of apple canker. The canker was on a limb about one inch in diameter and was about three feet long. Again on April 8, three

more isolations were made from a pear canker similar to the type just described on apple. One of these cultures produced infection when inoculated into young succulent apple shoots. Two of 26 cultures made from pear on April 29 also produced infection.

The results of these inoculations, with the date of isolation of the organism, host, and the region of the canker from which the isolations were made are given in table number 2.

Table 2

Results of inoculations with cultures obtained from dormant cankers

Date of isolation	No. of cultures obtained	Host	Region of canker from which isolations were made	Results of inoculations of young apple twigs with cultures from cankers
January 12	7	Pear		5+, 2−
February 6	5	4 Pear	¼ in. from edge	1+, 3−
		1 Apple		Negative
February 15	4	Apple	½ in. from edge	1+, 3−
*March 3	3	Pear	Center of small canker around spur	Negative
March 5	3	Apple	½ in. from edge	3+
March 8	3	Apple	12 in. from edge	3+
April 5	3	Apple	½ in. from edge	1+, 2−
April 8	3	Pear	12 in. from edge	1+, 2−
April 9	3	2 Pear	2 in. from edge	1+, 1−
		1 Apple		Negative
April 29	26	Pear	Advance of discolaration	2+, 24−

*The isolations on March 3 were from material collected at Ann Arbor in January and February which had been stored outdoors in the snow until the time of isolation as indicated in the table.

This table shows that out of a total of 43 pear cankers 10 harbored live fire blight bacteria and that in the case of the 15 apple cankers listed seven had viable bacteria present. The percentage of active cankers may have been higher earlier in the winter but it would be necessary to work with larger numbers before any conclusions on that point might be reached. It is probable that, occasionally, the organism may live over in the dead areas considerable distances back of the advancing edge of the canker especially in those of the indeterminate type.

Each spring during the course of this work, blighted orchards have been kept under observation for evidences of over-wintering of the blight organism in twigs and blossom spurs. Undoubtedly, the chief source of spring inoculum in an average season is from the cankers on limbs of more than an inch in diameter and from cankers on the trunk of the affected trees. There seems to be very good evidence however, as shown by Sackett (23) and later by Brooks (6) that the blighted twigs may in some seasons serve as a limited source at least of spring inoculum. The desirability of a full knowledge of the percentage of twigs in which the blight bacteria will live through the winter is evident since this has a direct bearing on practices of control by sanitation. Some of the evidence obtained during the course of this work indicates that twigs may occasionally be an important source of spring inoculum.

During the fall of 1926, a young apple tree was observed which had a number of blighted twigs. On May 23, 1927, these twigs were cut off and examined to determine the number in which over-wintering of the

organism had occurred. Since invasion of the healthy tissues in advance of the canker edge had already begun, examination was made microscopically. No isolations were made from this material. Table 3 gives the results obtained from examination of this material. This shows that over-wintering may occur in small twigs and it is possible that such twigs may occasionally serve as a source of inoculum in the spring.

Table 3
Over-wintering of the organism in twigs

Diameter of twig in inches	3/16	4/16	5/16	6/16	7/16	10/16
Number of twigs in which recent invasion was observed	4	1	3	5	3	0
Number of twigs in which no recent invasion was observed	0	18	0	6	0	2

Some further observations are presented here regarding over-wintering. A large Fall Pippin in the college orchard has been observed to have cankers on some of the large limbs during the spring months of 1927 and 1928. In the spring of 1927, the tree blossomed very heavily and about 90 per cent of the blossoms became blighted. Five or six cankers on the inside branches of the tree were observed to have become active about the middle of May in 1928. The few blossom clusters which were produced became blighted. Several isolated cases appeared in an adjacent crab tree. Numerous blossom infections also appeared on another neighboring tree. The blighted blossoms appeared on those limbs closest to the Fall Pippin tree.

Twig infection was observed near an active canker in a King in which no blossom blight had appeared. This canker was located about three inches above and to one side of the infected twigs.

One infection was secured on an apple seedling in the spring of 1928 from inoculum taken one-fourth of an inch back of the canker edge from the apparently dead bark of a Northern Spy canker. Another case was observed in the college orchard in which over-wintering had occurred in a twig seven-sixteenths of an inch in diameter. Evidence of this over-wintering was seen in the development of the canker which caused the death of the terminal bud at the end of the shoot shortly after it had produced leaves.

The fire blight outbreak in the pear orchard discussed on page 3, in which 20 per cent of the trees in a block of 323 were lost, originated from a single canker on a limb about one-half inch in diameter which was missed by the operator who was attempting to remove its blight cankers.

Observations were made in an orchard at Morrice, Michigan, the first week in July, 1928. In eight isolated trees, blossom blight had occurred. In seven cases, over-wintering had occurred in twigs no larger than a lead pencil.

It is quite obvious from the results of these observations and the observations of others that small twigs may at times serve as primary sources of inoculum and so the necessity of the removal of these twigs should not be minimized. The isolations of the writer also show that over-wintering of the blight organism may occur in dead tissue some distance back from the canker edge.

Dissemination

Various investigations of modes of fire blight dissemination show that there are two main agencies to be considered, insects and wind-blown rain. To control effectively the spread of the disease, the various factors concerned must be evaluated.

Dissemination by Insects

Among the insects listed as blight disseminators are bees, flies, wasps, the tarnished plant bug, (*Lygus pratensis* Linn.); aphids, (*Aphis mali* Fab.), (*Aphis avenae* Fab.); apple leaf hopper, (*Empoasca mali* LeBaron); curculio, (*Anthonomus quadrigibbus* Say.); shot hole bark boring beetle, (*Scolytus rugulosus* Ratzeburg); *Campyloma verbasci* Mey., *Orthotylus flavosparsus* Sahlberg, *Poecilоscytus basilis* Reuter, *Adelphocoris rapidis* Say., *Plagiognathus politus* Uhler.

It is evident that nectar seeking insects, during seasons of favorable weather conditions, are important factors in establishing primary centers of infection in the blossoming tree. The height of their activities is no doubt best recorded in the very severe blossom epidemics which are apparent from time to time. Observations by Waite (28), Crandall (8), Jones (16), Gossard and Walton (12) and others show that bees and other nectar seeking insects serve as the agents in bringing about blossom infection. Gossard and Walton (12) have also shown that infected blossoms may serve as sources of spread in a tree free from hold-over cankers.

These authors have also demonstrated that the organism may live in the honey in the hive 72 hours, which they believe to be about the limit of its viability under these conditions. Güssow (13) however cites data to show that the organism may live as long as 45 days when inoculated into sterile honey. Gossard and Walton (12) have evidence to show that bees may carry the organism in the pollen baskets as well as on the mouth parts. Blossoms of peach, plum, and cherry when inoculated with *Bacillus amylovorus* yielded the organism up to and including the fifth day. It is possible that blossoms so inoculated may serve as a source of inoculation for apple and pear if these trees be interplanted or in adjacent plots.

BLOSSOM BLIGHT

Blossom Infections by Nectar Seeking Insects, Field Observations During 1926

Experiments and observations of the writer on the matter of blossom infection by insects have been largely confined to dissemination by nectar seeking insects and by aphids. These experiments were carried on at the Huron Farms Company orchard at Ann Arbor and in the Michigan State College orchard at East Lansing.

During the early spring of 1926, the apple and pear orchards at Ann Arbor were very thoroughly inspected in an attempt to remove all blight cankers from the tops of the trees. The trunks and crowns of all the trees

were also examined. Three cankers were found after the trees had come into leaf. One was found in the pear block and two trunk cases were found in the apple block. Both of the trunk cases in the apples were just above the surface of the ground and accordingly little transmission, if any, was likely from either of these sources. The canker in the pear was in the top of the tree in such a location that rain which fell during the early part of the blossoming period could spatter from the canker to blossoms in the vicinity.

Because of past unproductiveness of the pear orchard in question, seven hives of bees were placed under the trees in row six. (Figure 1.) The hives were placed under every third tree numbering from the bottom to the top of the chart. A bee yard was established at the same time about 100 yards outside the main orchard, (Figure 2) opposite X.

The pear block consisted of Lawrence, Boussock, and Bartlett varieties. The trees represented in figure 1 are largely Lawrence and Boussock, the Lawrence predominating in rows 10-14 and the Boussock variety in rows 1-10. The first pear blossoms of the various varieties opened between May 14 and May 20. Precipitation occurred on May 14, 15, 19, 21, and 22. The Boussocks began to blossom on May 20 and rains of .04 inches on May 21 and .47 inches on May 22 fell during the early part of the blossoming period of this variety. The canker which was found in the pears was in a Boussock tree and it is very interesting to note that the heaviest blossom infection occurred in this variety.

A survey of about half the pear block, just after the first diseased blossoms became evident, showed many trees with blossom blight. After a thorough search, only one canker in a Boussock tree showed evidence of having been active before the main outbreak of blight took place, and this was located in the section of the orchard which had the severe infection. The evidence given in the diagram indicates that this canker was the original source of infection which took place. The tree in which the active canker was found it seen to be in the center of the severely blighted area. The squares or portions which are cross hatched represent 1 to 10 per cent of infected blossoms, the solid black represents 10 to 60 per cent infection. By far the heaviest infection occurred in the Boussock block and isolated Boussock trees showed quite heavy infection.

The widespread blossom infection which occurred in this orchard was undoubtedly due to the spread of the organisms from infected blossoms of the tree in which the active canker was found. Bees and other nectar seeking insects were undoubtedly the vectors concerned in the transmission of the disease.

Heavy infection from blight, following the usual course of fire blight, subsequently developed in this orchard as a result of this primary infection, as may be judged from the fact that it took two men about two weeks to remove the blighted parts from the affected trees over the whole 120 acres.

Field Observations, 1927

During 1927, additional field observations were made on this relation of nectar seeking insects to fire blight dissemination. On May 13 and 14, blossom inoculations were made by spraying a water suspension of the organism into the blossoms. These inoculations were made as a rule around the periphery of the tree and not over five or six feet from the ground.

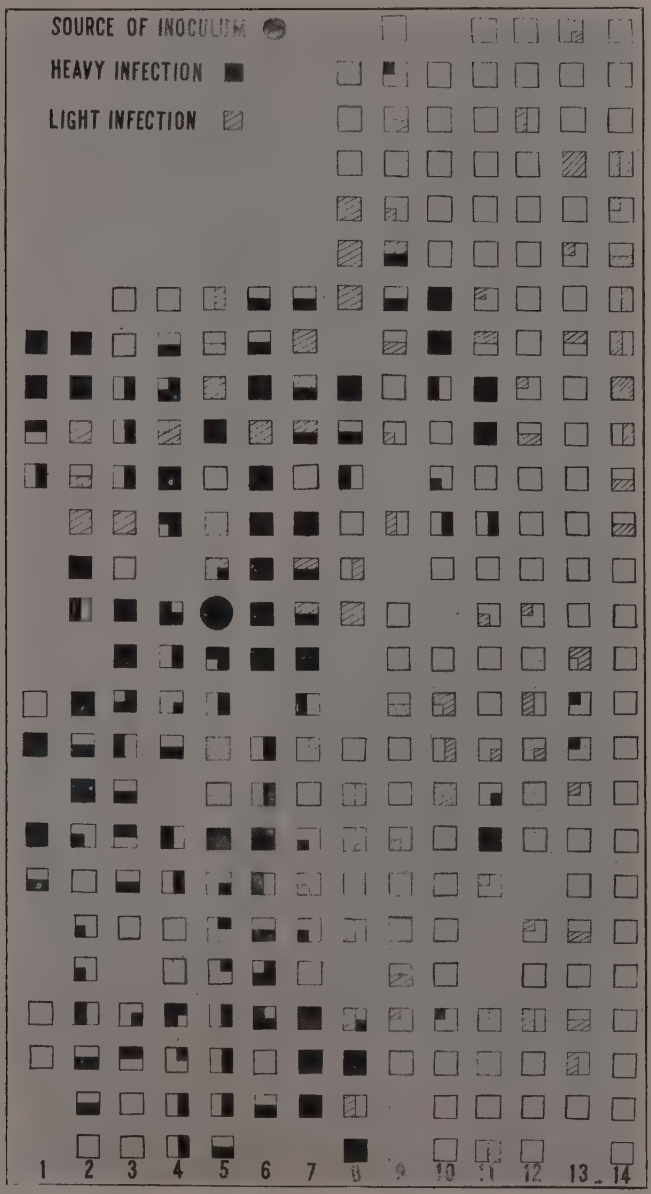

Figure 1.—Chart of part of the pear orchard of the Huron Farms Co. at Ann Arbor, Michigan. The chart shows the tree in which the holdover canker was found and degree of blossom infection in the other pear trees in this block of the orchard. Season of 1926.

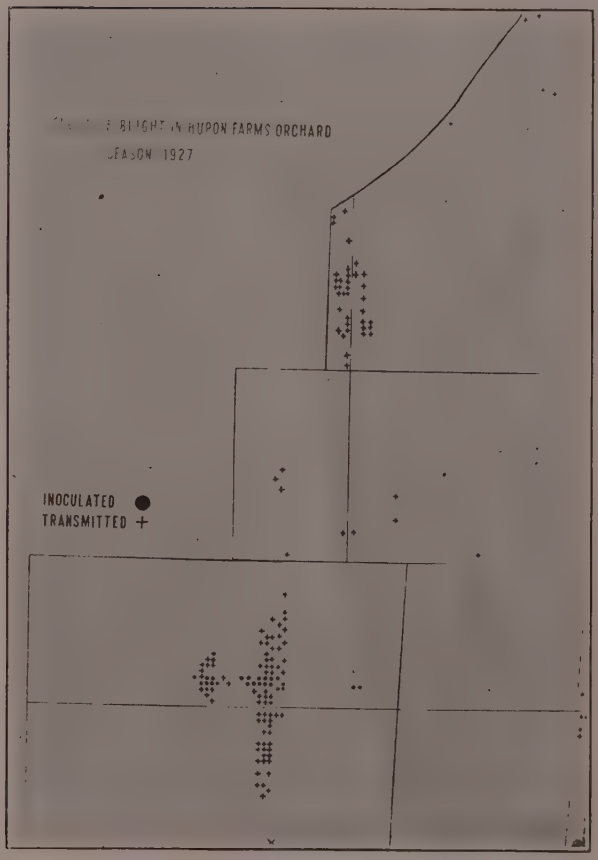

Figure 2.—Chart of the Huron Farms Co. orchard at Ann Arbor, Michigan, showing the amount of dissemination from known sources.

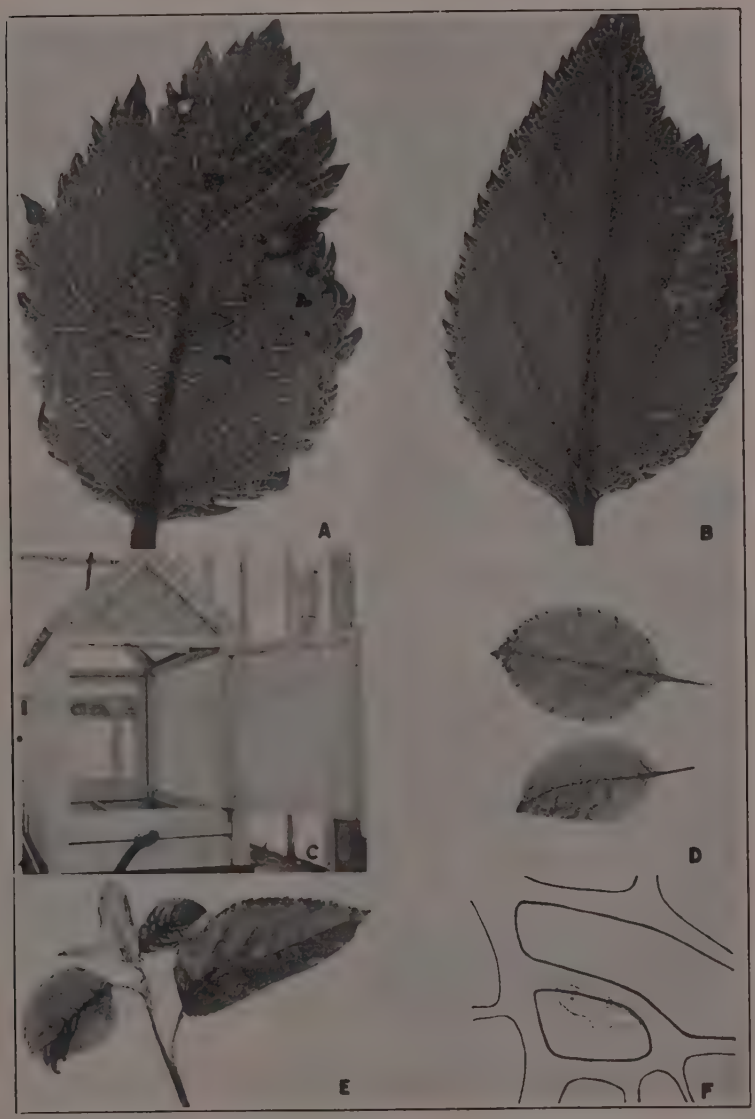

Plate I.—A. Young Borsdorf apple leaf. Ten lesions produced by stomatal infection. Note that the margins of the larger spots and all the smaller spots are much darkened. X7. B. Young Borsdorf apple leaf. Twelve lesions produced by stomatal infection. These spots were water-soaked but in this early stage no discoloration of the margin was evident. X7. C. Inoculation chamber. D. Leaf spot produced on Borsdorf by stomatal infection. E. Twig blight of Borsdorf resulting from stomatal infection. Note that the lower leaf also shows several leaf spots similar to those shown in figure D. F. Camera lucida drawing of earliest visible infection. This appeared as a small black speck by reflected light. The invaded area lies just below two stomata. X20.

Plate II.—A. Microphotograph of section of Borsdorf apple leaf showing earliest observed stage of invasion. The substomatal chamber and adjacent intercellular spaces, only, are occupied by the bacteria. X650. B. Microphotograph of section of Borsdorf apple leaf showing the advancing edge of the mass of organisms along a vein. X350. C. Microphotograph of tip of serration of young Borsdorf apple leaf showing the sequence of maturation of the tissues. Note that the cells of the lower epidermis to the left of the section are much smaller than those at the middle and right. Some of the cells to the left were still undergoing cell division. X575. D. Series of leaves described in table 7.

Table 4 shows the variety inoculated, the number of blossom clusters in each variety, and the number of these inoculations which produced infection. One blossom cluster was inoculated in each of the trees shown in table 4.

Table 4

Results of inoculating blossom clusters by means of a water suspension of B. amylovorus (May 13, 14, 1927.)

Variety	Blossom clusters inoculated	Blossom clusters infected
Rhode Island Greening	14	8
Wagener	17	7
Wealthy	17	4
Westfield	14	2
Grimes	7	1
Duchess	3	2
North Western Greening	5	3
Jonathan	2	0
McIntosh	2	1
Fameuse	6	4
Total	87	32

Nine of the 32 trees in which the blossoms were infected, showed further blossom blight. From these 32 possible sources, there were 110 trees infected. The writer had by examination of other apple and pear trees in the vicinity ascertained that no other sources of infection were at hand. Since some of the trees were a quarter of a mile from any other infected trees, it seems most probable that nectar seeking insects, probably bees, were the agents of transmission.

The weather during the blossoming period in 1927 was rather cool and a great many rainfall periods occurred during this time. The activity of bees and nectar seeking insects was no doubt below normal and this may have accounted for the rather limited amount of spread as shown by the survey (Figure 2). The largest losses occurred in the Wealthy variety, which was just coming into blossoming when these inoculations were made and was just opposite the bee yard. The heavy infection of the Wealthy trees, however, cannot be said to have occurred because of the fact that this was the only variety in blossom. It should be noted that the Jonathan, Northwestern Greening, Rhode Island Greening, Wealthy, and Grimes trees had, at this time, at least half of their blossoms open.

It has been noted that years of heavy blossoming are years when blight epidemics are likely to be severe. This was noted in the case of a Fall Pippin tree in the college orchard. Several cankers were observed in this tree in the fall of 1926 and again in the fall of 1927. The tree blossomed heavily in the spring of 1927 and about 90 per cent of its blossom clusters were blighted. Severe twig blight appeared also in this tree during the summer of 1927.

These cases, which have been carefully followed through the seasons of 1926 and 1927, leave little doubt of the importance of bees as agents for fire blight transmission.

Blossom Inoculation by Aphids

Some investigators have reported that aphids when hatching near the rough bark of the canker edge of fire blight cankers have crawled through the exu-

date and so have become contaminated with the blight organism. It is obvious that under such conditions these aphids so contaminated might crawl into the blossom and cause primary infection in the spring. The following experiments were carried on to determine to what extent the aphids might be of importance in this respect.

Healthy twigs on which large numbers of aphids were found were cut. These twigs were then allowed to wilt so that the aphids would cease to feed and start to crawl off the twig. These aphids were removed, by means of a camel's hair brush, to a moistened piece of bark from an active canker. They were allowed to crawl on this bark for 15 or 20 minutes so that their feet and perhaps mouth parts might become contaminated. Inoculations were then made by placing one such aphid on a style of each apple blossom. The results of these inoculations are given in Table 5.

Table 5
Inoculation of apple blossoms by contaminated aphids

Place	Blossoms inoculated	Blossoms infected	Blossoms not infected
College orchard	85	7	78
Huron Farms Company orchard	89	21	68
Total	174	28	146

This table shows that aphids may be the cause of primary infection in the tree, provided of course that conditions permit the aphids becoming contaminated with the pathogene. From the writer's experiments, it is concluded that aphids crawling into blossoms may inaugurate blossom blight, but the writer's observations have not indicated that this agency is of very much importance in the ordinary season.

Twig Infection by Aphids

For several years it has been generally believed by fruit growers as well as by a number of plant pathologists and horticulturists that aphids are very important in spreading blight from tree to tree. It did not seem at the beginning of the investigations reported in this paper that the evidence presented by Jones (16), Merrill (18), and Burrill (4) was extensive enough to justify very definite conclusions regarding the role played by these insects.

Inasmuch as wind blown rain is a factor in dissemination of the causal organism as was shown by Stevens (25), Gossard and Walton (12) and others, the results obtained by Burrill are open to criticism. His experiments were carried on in the field and he reported that a twig outside of his cages became infected, so it is entirely possible that infection was present in the trees in the vicinity of the inoculated shoots. He does not state that infection was not present. The infections secured might presumably have come from the outside, especially if rain had fallen, irrespective of the aphids.

Merrill's experiments were made in the open and he notes that rain fell during the period on which the inoculations were made. The aphids then might have been only accidental carriers of the organism and not active in the production of infection.

The importance of this question from an economic standpoint alone would seem to justify the expenditure of considerable effort in an attempt to evaluate the importance of these insects in the spread of fire blight. With this idea in mind, the following experiments regarding aphid transmission were carried out during the summer of 1927 and the spring of 1928. Both green and rosy aphids were used in making these inoculations.

Green aphids (*Aphis mali*)

Three aphid-covered, blighting twigs of a Wagener tree were observed in the Huron Farms Company orchard, on August 1, 1927. The signs of the disease had just begun to appear on these twigs even though exudation had occurred six inches back from the tip of the twig. The aphids at the time of removal from the twig had ceased feeding and were crawling around. It has been observed that under orchard conditions aphids do not remain on the diseased portions of the twig after the tissue becomes water soaked or browned. The aphids on healthy twigs were not crawling about. The aphids on the infected twigs were transferred to three succulent healthy twigs of the same variety. No disease appeared in these twigs. On August 9, aphids were removed from blighting twigs of Winter Banana, and were placed on 15 rapidly growing, healthy shoots of the same variety. Five aphids were placed on each shoot. At the same time aphids were removed from similar blighting twigs of a Rhode Island Greening tree and transferred to 35 healthy shoots of the same variety. Five aphids were placed on each shoot as in the previous experiment. The exposed twigs in both varieties remained healthy.

On July 9, 1928, an experiment was carried out at the nursery of the Horticulture Department of the Michigan State College in a further attempt to determine how efficient aphids might be in transmitting the disease to twigs. Twigs infested with green aphids were brought into the laboratory where they were allowed to wilt. After the aphids had crawled off they were allowed to crawl through a water suspension of the organism. Fifteen aphids were then placed on each of 120 succulent Borsdorf seedlings. Forty twigs of the same variety were used as checks to determine the relative susceptibility of the twigs used in the experiment. These were inoculatd on July 11. Twenty of these were inoculated by puncture and on 20, a water suspension was put onto the tip leaves and the stem. These checks were inoculated during a rain which lasted several hours. Three weeks after the inoculations were made, two of the aphid inoculated shoots and nine inoculated by puncture became blighted, the rest remained healthy.

Rosy aphids (*Aphis sorbi*)

Similar experiments were carried out in the spring of 1928 in the greenhouse and in the nursery at the Michigan State College. The rosy aphid was used in all these experiments.

Twigs infested with rosy aphids were brought into the laboratory and allowed to wilt. After the aphids had crawled off, they were placed on blighting shoots of Transcendent Crab from which the leaves had been stripped. The blighting shoots were kept in water and the aphids transferred to them by means of a brush. The aphids were then placed on the foliage of potted Borsdorf seedlings kept in a wooden case in the greenhouse to make certain that the foliage would not be accidentally watered. The aphids crawled off the blighted twigs and colonized on 14 of the Borsdorf shoots. At the end of 20 days, no blight developed on any of the plants. Seven days

is the usual time required for symptoms of blight from puncture inoculations to become evident under the conditions of these experiments.

Other aphids were allowed to crawl through a suspension of the organism in water. The suspension was made from macerated twigs of Borsdorf and Transcendent Crab. On each of 22 shoots of potted seedlings, five of these aphids were placed. These colonized and produced young. These plants were also put into the glass chamber in order to have the foliage remain free from water which might otherwise fall on the leaves in watering the other plants in the greenhouse. These plants were free from blight at the end of 20 days.

A similar experiment was carried on in the nursery of the Horticulture Department on Borsdorf and Ben Davis seedlings. Three series of 15, 80 and 55 twigs, respectively, were exposed to inoculation by placing five aphids on each twig. The aphids used had previously been allowed to crawl through a suspension of the fire blight organism. These twigs were all bagged with glassine bags to keep out rain. After three days the bags were removed and the aphids killed with a nicotine solution.

Two days later, 450 more tests were made in the same manner as described above except that the twigs exposed to the aphids were not bagged and the aphids were killed about 24 hours after they were placed on the plants. One twig of the second series of bagged twigs became blighted on July 2.

In all, about 4500 aphids were used in these experiments and about 800 twigs were exposed to the aphid attack. Six check shoots which were inoculated by puncture on June 20 became blighted at the end of 10 days. Because of the few positive cases, it cannot be said as a result of these experiments that aphids are not a factor in the dissemination of the disease to twigs. It is however, quite evident that under the conditions of the experiments, little infection was inaugurated by aphid punctures of growing twigs. Two hundred aphids were put upon one seedling to determine if possible whether the number of aphids used might influence the results, but this seedling also remained healthy. The results of these tests seem to indicate that under ordinary conditions, at least, aphids are not important factors in bringing about twig blight.

Dissemination by Meteoric Water

The importance of meteoric water as a means of dissemination of bacterial diseases of plants has been quite thoroughly worked out in the case of some diseases. This agency of dissemination may be quite important, and, for the eastern portion of the United States, it is true that in the average season it is the most likely to function of any of the factors which might bear on the dissemination of the blight organism. It has been noted by numerous investigators that periods of blight infection may often be closely correlated with rainfall periods.

Stevens, Ruth and Spooner (25) found that wind was a factor in blight dissemination, Faulwetter (11), working on angular leaf spot of cotton, Rolfs (21), on black spot of peach, Wolf (30), on citrus canker, Rapp (22), Sackett (23), and Edgerton (10), on bean blight, and Carsner (7), on angular leaf spot of cucumber all suggest that meteoric water may be an important factor in the spread of these bacterial diseases. Some of the investigators mentioned have found rain and dew to be important factors in disease production, and others suggest meteoric water as a possible means of spread.

Because of the fact that insect transmission has been stressed in the past and because of the prevalence of the suspected insect vectors in the orchard

in any normal season, exact data on spread of fire blight by wind blown rain or rain drip under conditions where insect agency is eliminated are difficult to secure under field conditions. Observations were made in the early summer of 1926 at Ann Arbor and in the field at the Michigan State College in the spring of 1928 in an attempt to determine how important rain may be in dissemination of the fire blight organism.

The first symptoms of blight appeared on June 14, 1926, in the pear planting of the Huron Farms Company orchard, and, by June 16, the symptoms had appeared in abundance in the apple trees adjacent to the pear trees. On June 14, 1.03 inches of rain fell and .09 inches fell on June 15, with .22 inches on June 21 and .07 on June 25. These rains should have been sufficient to spread the organism from the blighted blossoms to the twigs. It should be noted too that, while a few rosy aphids and leaf hoppers had been observed on May 30, the orchard as a whole was almost free from these insects until well along in the summer when green aphids were present but not in sufficient numbers to justify the use of nicotine to control them.

Summer cutting out of blight in the apples was begun during the last week of June and during its progress it was observed that, in the trees nearest the pears, twig infection often occurred beneath infected blossom clusters. A record was kept of the instances in which these infected twigs were observed to occur beneath infected blossom clusters. On the first day, 41 cases of twig blight were found beneath infected blossom clusters. On the second day, 11 cases of blossom infection alone were found and 38 cases of twig infection below blighted blossoms. Eleven blighted twigs were found during this period in trees which had no infected blossoms; these trees were adjacent to trees in which blossom blight occurred and it would not be impossible as shown by Faulwetter (11) that wind blown rain, or even spatters of rain from infected blossoms of neighboring trees, might have been the agency of transfer of inoculum. The twigs when removed were blighted back 10 to 12 inches from the tip which is about average distance for twigs two weeks after inoculation, as compared with conditions found in experimental inoculations.

The following observations were made on some small Transcendent Crab trees at the Michigan State College. Nine, seven, 15, and 16 blighted twigs, respectively, had been removed on May 18, from four trees. On May 25 these trees had five, seven, two, and five infected twigs respectively. Rain had occurred during the week of May 11 to May 18. No aphids or leaf hoppers were found when a thorough examination was made of these trees. Neighboring trees of the same age and variety in which no blighted twigs occurred remained free from fire blight during this test.

Other tests were made in which blighted twigs were fastened into the tops of some of the young apple and pear trees. These trees were then sprayed over night by an oscillating overhead irrigation outfit. The trees had also been sprinkled before the inoculations were made. Three twigs of one pear tree so treated became blighted after 20 days. Four twigs of one apple tree and one of another of three similarly inoculated trees became blighted. Blight occurrence in this test was restricted to the trees having the blighted twigs placed in the tops.

From the results of observations during the past three years regarding twig infection, it seems that a close correlation exists between rainfall periods and the appearance of twig infection. The infected blossom clusters, in the case of observations made in 1927, left no doubt as to the source of primary

twig infection. These infected twigs served as a further source of inoculum for twig infection during the summer. It has been observed that higher percentages of twig infection occur in trees which have severe blossom blight. It seemed hardly probable to the writer that insects served as carriers for this primary twig infection as they do not attack the less succulent tissues such as are found in the blighted blossoms and blossom spurs.

INFECTION[1]

The Relative Importance of Wounds and Natural Openings

The work of various investigators on infections of the apple and pear by the blight organism has raised the question as to the relative importance of wounds made by insects, man, wind, and other agencies, and of the natural openings in the plant tissue. It is a well known fact that usually both types of infection occur in any blighted orchard, yet which of these may be the more important is difficult to determine.

Blossom infection occurs through the nectaries of the flowers, and blossoms which have been inoculated by spraying a water suspension of the organism into them soon exhibit signs of the disease as has been shown in the tests described previously. It was observed that these blossoms showed water soaked spots in the calyx cup after three to four days, and, on the second or third day following, these spots had become dark brown to blackish in color. On about the tenth or twelfth day, a water soaked condition could be seen on the outside of the calyx cup and drops of exudate appeared several days later.

The organisms are at first located among the cells of the nectary, but, as they multiply, they spread throughout the tissue of the torus and ovary and then push downward into the pedicel through the intercellular spaces. Invasion of the fruit spur follows and many times the young fruits are invaded from the spur through the pedicel.

In the apple, the invasion of the spur usually results in canker formation at the base of the spur, especially on the more susceptible varieties. In the pear, if conditions are at all favorable, the spur infections also usually result in invasion of the limb. From this invasion, the scaffold parts become involved and large limb cankers or even body blight develop.

Twig infection as reported by numerous investigators occurs near or at the tip of the twig, at least the symptoms appear first in this region. Further invasion by the organism involves the basal part of the twig and the bark of the limb in the immediate vicinity of the base of the twig. In many cases, the progress of the organism is checked at this point at least temporarily, and a quite definite line appears, due to the formation of a large amount of cork by the host tissue. Often, however, the organism is not entirely cut off from the bark tissues by this layer and further invasion follows. These periodic waves of advance leave more or less distinct ringed markings on the bark. These concentric markings are often even more marked in the inner than in the outer bark.

[1]Since the completion of this manuscript for publication, there appeared in Science, n.s. 68: 386-388. Oct. 19, 1928 an article by Paul W. Miller entitled "A preliminary report on studies of fire blight of apple." In this paper the following subjects dealt with in this bulletin are discussed: (1) the role of meteoric water in the dissemination of the primary inoculum, (2) the infection of young shoots without the intervention of insects, (3) the stomatal penetration of young apple leaves, (4) the over emphasis in the past of the role of insects in fire blight dissemination.

Insect wounds are often found to be the seats of infection in the fruits. Several investigators have recorded this for curculio wounds and 12 such cases were observed in the Huron Farms Company orchard in 1926. No doubt injuries caused to the fruit by codling moth larvae likewise serve as paths of invasion. Wounds on leaves caused by the wind whipping them against fruits and branches also may serve as places of entry. Six cases were observed in the spring of 1928 on small Transcendent Crab trees in which mature leaves became infected at such injuries, and twig blight resulted in all the cases.

Limb injury caused by pruning or the removal of blight infected twigs during the growing period frequently admits infection. Injuries to limbs and crotches brought about by pickers have served as a place of entrance of the organism. Wounds on the trunks from saw cuts, from removal of water sprouts, and injuries from disks or harrows in cultivated orchards frequently become infected and start trunk cankers. An orchard of pears near Sodus, Michigan, which was examined in the early summer of 1928 proved to have very nearly five per cent of the 2000 trees infected in the trunk, and the majority of these cases could be traced to disk injury on the trunk. Cultivation too close to the tree is also very likely to wound the roots and infection through these wounds was plainly responsible for the death of trees in many cases.

Ten 18-year-old pear trees at the Huron Farms Company orchard were inoculated at the crown by wounding the tissues in this region with a spade; only two or three wounds were made on the crown of each tree. Two gallons of a water suspension of the organism was poured around each of these trees. Two of the trees became diseased through these wounds. It will be noted in this recital of wounding in relation to blight occurrence that the wounds may vary widely in origin, and the tissue concerned may be of any age or kind. If the organism is transferred to a wound, blight may result. In quite another category, must be placed the matter of twig infection, for here wounding is not apparent.

Twig infection, on the whole, has not been, it seems to the writer, satisfactorily explained. Reports by various investigators of severe twig infections in the absence of any of the insects which have been reported to transfer the causal organism, raise the question as to what additional factor or factors may be concerned. Some experimental evidence by Merrill (18) and Burrill (4) seems to show that aphids may be a source of spread, as has been pointed out before, although experiments with about 4500 green and rosy aphids on 800 shoots, carried out at the Huron Farms Company orchard and at the Michigan State College orchard, have failed to indicate that aphids are important agents for infection since only three twigs became infected.

Some inoculations made in the greenhouse during the early winter of 1927, on plants which had not been wounded and on which no insects could be found, led to infection. The question arose as to how these infections had occurred. The first observed symptoms in these plants was a browning of the tips and discoloration of the tip leaves of the infected shoots.

Burrill (5) in 1880 writes of twig infection, "the leaves, however, are invaded from the bark, or as it seems may be the starting point of the infection. I have no direct experiment upon this point but have found infected and dying leaves on healthy stems." Experiments in which he painted the under side of the leaves of apple and pear with a suspension of the organism gave only negative results.

Arthur (1) in 1885 reported that the tips of apparently healthy young pear leaves became infected when smeared with a culture of the organism. Stedman (24) in 1893 suggested that organisms carried by wind blown rain or perhaps by insects to the leaves entered through the stomata. Duggar in his text[1] makes the following statement regarding twig infection: "Nevertheless, it is also true that infection may result through growing twigs, injuries and sometimes perhaps even water pores may be the seats of infection. In general, however, it is certainly true that the germs upon the surface of healthy tissues would not result in the production of disease in those parts."

Heald (15) in 1915 reported that at North Yakima cases were observed in which lesions of Bartlett pear leaves were found advancing from the edges or tips of the leaves. Such lesions on the following morning after a rain showed drops of bacterial exudate. Similar lesions were found a few days later in the vicinity of Spokane on apple leaves. Further collections of similar material were made at Walla Walla, Kennewick, and Prosser from both pear and apple trees. He observes that the initial infections "in the majority of cases start at the margin and are either lateral or terminal although central lesions have been found in some cases on apple leaves." Brooks (6), working at Wisconsin, reported that he was unable to secure stomatal infection.

Experiments were carried out at East Lansing to determine the nature of infection in these leaf lesions and the points of origin of the infections. Borsdorf seedling apple trees about three years old were used. Other varieties, Ben Davis, Winesap, and Jonathan were used in later experiments with the same results as with Borsdorf.

Potted seedlings were placed in the inoculation chamber as shown in Plate I, Fig. C. Five or six seedlings were inoculated in this chamber at one time. The plants were first sprayed by means of the spray attachment in the inoculation chamber to wet the leaves thoroughly both on the upper and lower surface. The organism was then sprayed onto the under leaf surface in a water suspension by means of a DeVilbiss atomizer, or small pieces of diseased tissue about one millimeter square were placed in the droplets of water which collected on the under side of the leaves.

Inoculation on the upper side of the leaves gave negative results except in those cases in which the immediate tip of the serration was included in the drop of water in which the inoculum was placed.

The plants were kept in the inoculation chamber for 20 to 24 hours, and the leaves were kept wet by occasionally turning on the spray. The attachment was turned at an angle so that only a fine mist filled the inoculation chamber without the spray striking the leaves directly. The plants were then removed to the bench and examined several times daily for the appearance of the symptoms. There were 124 plants inoculated in this series of 32 inoculations. The inoculations were made over a period of approximately one year.

The percentage of infection ranged from 0 to 100. It is possible to explain the failure of many of the plants to become infected. In some cases, the temperature of the greenhouse was so high that it was not possible to keep the leaves of the plants wet for more than a few hours at a time and in this event no infection resulted. Inasmuch as the time required by the organism to gain entrance into the host under various environmental conditions is not known, it is possible that the failure to secure infection was caused by the drying off of the leaves while the plants were in the inoculation chamber. It was observed that the period of incubation varied from two to five days depending

[1]Fungous Diseases of Plants. 1909.

on greenhouse conditions. The symptoms appeared in shortest time when the greenhouse was very warm.

The lesions when first noticeable on the leaves are minute, black dots which are visible only by reflected light. Within three or four hours these areas increase several times in diameter and have a water-soaked appearance. Soon they occupy the area between adjacent veinlets making an angular spot. Plate I, figure B represents a leaf in which the spots have reached the water-soaked stage. Shortly after, the edges of the spot become browned and the progress of the organism is checked as these veinlets are reached as shown in Plate I, figure A. It is possible that the arresting of the organism at this point is mechanical as the intercellular spaces of the tissues surrounding the veins are very small in comparison to the size of those of the mesophyll. Within several hours after penetration of this tissue, the water-soaked condition appears along one of the veins leading to the midrib and the invasion proceeds down this vein to the midrib as evidenced by the discoloration of the tissues in this region. Once the midrib is reached, the invasion downward along the midrib follows. This advance may involve the midrib and the tissues on both sides or it may be restricted to the tissues on one side of the midrib. The petiole invasion occurs upon the under side, and the infection passes into the twig or spur. Plate I, figure E shows such cases of invasion on Borsdorf seedlings. Invasion of the shoot has been observed to occur within 24 hours after leaf infection became visible under greenhouse conditions, although four days is the shortest time found under field conditions.

Leaf spot may also result from leaf infections as is shown in Plate I, figure D. These spots showed no further development after three weeks.

Since the results of the inoculations on Borsdorf and other seedlings showed no varietal differences, no attempt is made to differentiate between the varieties in recording the results in Table 6, which gives the results of these inoculations as obtained in the greenhouse during the winters of 1927 and 1928.

Table 6 shows that 57 per cent of the 124 seedling trees which had been inoculated without wounding became infected. Careful examination of the infections secured showed that in only one case was infection secured near a wound of any kind.

The largest number of infections on a single leaf was 28 and the average number in the different series varied quite widely with different conditions in the greenhouse at the time the plants were in the inoculation chambers.

The green peach aphid was present in considerable numbers on some plants which were inoculated. However the wounds produced by these insects in feeding are very characteristic and may easily be determined by use of a hand lens or microscope. Only one case in over 200 lesions examined was observed in which infection might possibly have occurred through one of these wounds. A series of inoculations was also made on aphid free plants and is so noted in Table 6.

Inoculations

Field inoculations were made on Transcendent Crab and on pear. The plants to be inoculated were first sprayed by means of an oscillating spray for varying lengths of time. The inoculum was put upon the young rapidly growing twigs in an aqueous suspension. One or two drops were used for

Table 6
Leaf inoculation of apple seedlings* without wounding

Date of inoculation	Plants inoculated	Plants infected	Per cent of infection	Remarks
1927—				
March 3	9	7	77	
March 7	5	3	60	
March 15	3	0	0	
March 20	2	2	100	
October 17	3	0	0	
October 24	1	1	100	
October 25	4	2	50	
October 28	4	2	50	
October 29	4	2	50	
October 31	4	0	0	
November 1	1	1	100	
November 2	4	0	0	
November 8	2	2	100	Pure culture
November 10	3	1	33	
November 13	3	1	33	
November 17	2	1	50	
1928—				
February 7	4	2	50	
February 8	4	0	0	Samples taken for sectioning
February 9	4	2	50	
February 11	4	2	50	
February 18	4	1	25	
March 1	4	3	75	
March 7	5	5	100	
March 8	2	2	100	
March 10	4	4	100	
April 18	3	2	66	
April 24	3	1	33	Aphid free
April 30	4	4	100	Aphid free
May 1	5	4	80	Aphid free
May 4	5	4	80	Aphid free
May 6	4	3	75	Aphid free
May 10	11	7	63.6	Aphid free
Total	124	71		

*Seedlings used in 1927 and in 1928, except where indicated as "aphid free" were the ordinary greenhouse grown seedlings and a few aphids occurred from time to time. See discussion in text.

each twig. The spray was then turned on again and allowed to run over night. These plants were apparently free from aphids and leaf hoppers at the time the inoculations were made.

The field inoculations, in which four small Transcendent Crab trees were used, gave 24 per cent positive inoculations of a total of 273 inoculated twigs. A similar set of inoculations the next day on three trees with 89 susceptible twigs gave only one positive inoculation.

Of 200 inoculations on pear twigs, only five twigs became infected. Another set of inoculations of seven pear and 17 Transcendent Crab trees gave no positive results except where the organism gained entrance through an injury on the apple leaves, 10 such cases occurring.

It is possible that the treatment before inoculation was the factor influencing this divergence in the results. The plants which gave the highest percentage of infection were sprayed for five hours before inoculation. In the experiment the following day, in which about one per cent infection was secured, the plants were sprayed for only one-half hour before being inoculated.

In the last experiment, due to the fact that the trip mechanism of the

sprinkler failed to work, only three trees were sprayed very thoroughly before being inoculated.

The evidence on insect transmission as presented by the various investigators does not seem adequate to explain twig infection under the varied conditions after which infection appears. As has been pointed out, twig infection frequently occurs in the absence of insects which are supposed to transmit the organism. It is of course entirely possible and indeed it is very probable that some of these infections take place through wounds other than insect punctures or bites on the leaves and stems.

Examination of early stages of leaf invasion have shown that infection occurs on healthy sound leaves and these infections occur through the stomata of the leaf while it is in certain definite stages of development. It seems quite likely that this type of infection occurs in a large percentage of the observed twig infections in this state.

Examination of Prepared Material

Microtome sections of the various stages of leaf infection proved that infection was without question through the stomata as was suggested as a possibility by Heald (15). Plate II, figure A is a photo-micrograph of a cross section through a young leaf. It will be noted that the sub-stomatal chamber alone is filled with bacteria. A little later stage of infection is shown in Plate I, figure F, which is a camera lucida drawing of the surface view of a spot which has just come to occupy the area beneath two stomata. This appeared as a very small water-soaked spot on the leaf.

Invasion then proceeds downward through the intercellular spaces of the mesophyll along a vein. These intercellular spaces are quite large and the movement of the zoogloeal mass is quite rapid. Invasion of the petiole occurs through the mesophyll-like tissues of the under side of the petiole. It has been noticed that invasion of the petiole frequently includes tissues on one side of the mid vein only, which is itself often not invaded.

Sections have been observed in which the advancing edge of the zoogloeal mass is wedge-shaped and tapers off rather gradually. The advance is always along a vein and it is quite possible that the organism moves in this region because of the fact that more water is available. Plate II, figure B, shows the advancing edge of an invading mass of bacteria on the left side of the vein.

It is of interest to note here that the host cells, in the early stages of invasion by the mass of bacteria, showed no apparent signs of death even when the spot had reached the stage in which discoloration had begun to show around the edge of the spot, the only sign in the host cells being the apparent shrinking of the cells of the lower epidermis. However, by the time the organisms had begun to invade surrounding tissue or had reached a vein, the mesophyll and lower palisade cells in the region first invaded showed a slight plasmolysis and also a tendency to take the stains more readily than did the healthy cells in the surrounding tissue. When pronounced browning had occurred the cells appeared to be quite badly plasmolyzed and the chloroplasts in particular stained very heavily. Very little invasion of the palisade region occurred.

Migration of the Organism

A study of the migration of the organism in the leaf and stem tissue was also made in connection with the production of twig blight from in-

fections in the leaf. The findings of Nixon (19) were corroborated in this study. The matrix in which the organisms are embedded is jelly-like as Nixon describes it, and more dense apparently than Miss Bachmann (2) believed it to be. She concluded from her studies that invasion was accomplished by the movement of the organism in a liquid which she believed to be withdrawn from the lumina of the adjacent cells causing them to lose their turgidity.

Stained microtome sections of infected leaves showed the organism to be embedded in a substance which stained very definitely with most of the common stains such as gentian violet, acid fuchsin, eosin, iron alum haematoxylon, Delafield's haematoxylon, methylene blue, and licht grün. Nixon used Fleming's triple stain and the matrix then stained a light blue with the organisms a dark red color. He refers to these masses as zoogloeae, and in this interpretation the writer concurs. Beijerinck (3) and other investigators have demonstrated that certain bacterial organisms can build up from the simpler sugars certain pectic compounds, and, from a few tests, it seems that the matrix is pectic in nature. Microtome sections of diseased leaves when stained with ruthenium red, a specific stain for pectin-like substances, give a positive reaction in the matrix, while other cellulose tissues fail to take the stain. That diseased twigs have a decidedly higher sugar content than normal twigs was shown by the increased reduction of Fehling's solution with equal amounts of macerated healthy and diseased tissues. The tests as made were not quantitative or specifically qualitative but may indicate in a measure at least the nature of the matrix.

Normal Anatomy of the Apple Leaf

During the experiments on infection of apple seedlings it was observed that a rather close correlation existed between the age of the leaf and the degree of susceptibility to infection and it seemed that a study of the normal anatomy of the apple leaf might aid in interpretation of some of the results obtained by the inoculations.

Superficial microscopic examination and microtome sections showed that the leaf as it emerges from the bud is composed of a meristem-like tissue in which the veinlets and veins have been differentiated. As it increases in size, the palisade, mesophyll, and epidermis at the apex begin to mature. The increase in the size of the cells of the upper epidermis is accompanied by an increase in the size of the palisade cells. On the lower surface, stomata are formed by the division of epidermal cells, and shortly after, the formation of the intercellular spaces of the mesophyll occurs. Hydathodes or stomata which are slightly larger as a rule than the normal stomata may be found on the upper surface of the leaf at the tips of the serrations. There may be as many as six or eight of these but usually not more than three or four. These hydathode-like structures develop very near to the end of the vascular strand which terminates in the serration tip.

Plate II, figure C, shows the maturation of the tissue at the tip of a serration which is entirely comparable to the condition observed in the leaf tip proper. Stomata are shown in various stages of development, the youngest being the most distant from the tip. An increase of size of epidermal cells was noticed in the region of the mature stomata.

The guard cells, as soon as formed, increase rapidly in size and, in so doing, tend to bow outward forming a substomatal chamber. The subse-

quent development of the leaf allows the guard cells to assume the normal position which is approximately level with the surrounding epidermal cells.

The maturation progresses from the tip of the leaf and the tip of the next lower serrations downward and inward to the base of the leaf. Plate II, figure D shows a series of leaves taken from a young shoot. Table 7 gives the length of leaf, approximate age in days, and the region in which stomata are found in this series of leaves. The determination was made microscopically.

Table 7
Leaf development of apple

Number of the leaf counting from tip downward	Length of leaf in mm.	Approximate age in days	Distance Stomata found downward from tip
1	8	1–2	½ mm.
2	10	3	1 mm.
3	16	4	2¼ mm.
4	22	7	5 mm.
5	35	10	13 mm.
6	45	14	Whole leaf

This study furnishes a plausible explanation of the fact that the success of inoculation was found to depend upon the age of the leaf or of the portion of the leaf inoculated. No infection was secured on very young leaves whose stomata had not as yet developed. Successful invasion into the twig was secured only through infection of young leaves whose stomata had become functional. The zoogloeal mass in these leaves was able to penetrate the tissues rapidly and reach the mid vein of the leaf. If the leaf had developed to the stage in which the intercellular spaces of the mesophyll were formed but were as yet small because the cells had not become mature, only a leaf spot resulted since the infections were quickly delimited. As soon as the leaf or any part of it was mature, that leaf or part of the leaf was not susceptible.

These stages may be quite easily determined and this may aid in practical methods of control as trees in which no new shoots are found may be regarded as not susceptible to twig infection in the manner just described. The mature region of a young apple leaf may be quite accurately determined by the color. The mature region is dark green, the immature region is a greenish yellow. The mature region shows microscopically, mature stomata and intercellular spaces in the mesophyll. The immature region is composed of small, closely compacted cells which in section are cubical to slightly round.

It was shown by this study that the maturation of the tissues of the apple leaf is progressive from the tip and serrations downward and inward to the petiole. This fact explains why apple leaves in their development are susceptible only during certain stages. The following relation between infection and leaf development was found.

1. Leaves too young for infection. No stomata developed.
2. Leaves susceptible. Stomata functional. Substomatal chamber formed.
3. Leaves susceptible. Intercellular spaces of the mesophyll formed but not so large as in the mature leaf. Such infections produce only leaf spots.
4. Leaves not susceptible. Tissues mature.

Standard Control Measures

Control measures recommended for fire blight are sanitation, the use of resistant varieties, and the practice of proper cultural methods. Exclusion of the organism by means of sprays has not yet been proved to be of any great benefit.

Crop sanitation in the form of removal of diseased parts is at present the most practicable control method for Michigan. Removal of diseased limbs and cankers may be done either during the dormant or growing season or, as is practiced by some growers, during both the dormant and growing seasons.

The time of removal of the diseased parts must necessarily be determined by the conditions existing in the orchard. It has been recommended that under Michigan conditions removal may, usually, be best accomplished when the trees are pruned during the dormant season. If few cankers are known to be present in the orchard, the removal of these cankers not only is a small matter but also insures the grower against the possibility of a severe blossom infection the following spring, as often occurs.

In heavily fertilized orchards, occasionally, the spread of infection following heavy blossom blight becomes unusually severe and the removal of diseased parts during the growing season seems desirable. This practice has a more important place in pear than in apple orchards because of the more rapid development of fire blight lesions in the pear tissue. The possibility of spread by the tools used in removal of the diseased parts is much greater in the case of the pear than the apple and this means that the operator must exercise great care in the removal of diseased parts. With the exception of very late falls during which much precipitation occurs, late infections in the apple in this State are not of much importance.

The use of resistant stocks, which at present is still hardly practicable due to the scarcity of material of the varieties recommended, may eventually be of considerable value so the trunk and scaffold parts of the trees will be saved in case of infection. It is true that, even though this part is saved, severe infection will mean the loss of the greater part of the top in many cases. The necessary top working may reduce the productiveness of the tree for a period of years.

The increased cost of the trees when they are introduced commercially will probably be a factor which will discourage many growers from planting these trees worked on resistant stocks. Other such cases have been recorded and there is no reason to believe that this may be an exception. Reimer's* work does show that some of these varieties hold considerable promise for use as stocks for root and top working. At present no very resistant apple varieties are known which might be used for the scaffold parts.

The present practice of some growers of using susceptible varieties of apples as fillers in the orchard is of questionable value. Similarly, the maintenance of the crab apple, and such varieties as Yellow Transparent and Tolman Sweet near blocks of other varieties augments the fire blight control problem.

The Wealthy and Duchess are very susceptible to fire blight once infection occurs. The value of the Wealthy as an early apple in this State, however, overbalances to some extent its undesirability due to its blight sus-

*Reimer, F. C. Blight resistance in pears and characteristics of pear species and stocks. Oreg. Agr. Exp. Sta. Bul. 214: 1-99. 1925.

ceptibility. According to the writer's observation, the Duchess, even though susceptible, is seldom severely infected in Michigan, which may be due to its early blossoming habit.

From observations of orchards in the past three years the advisability of planting Wagener trees as fillers is questioned, as the tree is very susceptible to infection. Crown and root infections were observed to have been very abundant in the Huron Farms Company orchard on this variety and many trees had to be removed on account of severe injury from blight.

Cultural methods may go far to aid in control of the disease. Most Michigan orchards unless fertilized or heavily cultivated do not make much late growth and so are not subject to late twig infection. Twig cankers from these late infections in the apple, it seems, are one source of over-wintering of the organism. Due to the differences in rate of invasion in apple and pear the apple rarely becomes infected in the scaffold and trunk from blossom or twig infection unless through spurs or shoots on these parts, while infection at the tips of branches or branch spurs of pear often results in invasion of the larger limbs and trunk.

Late growth if allowed to develop will serve as a source for further infection and so successive waves of twig blight will carry active canker formation over into the late summer and fall. Quick early growth with terminal bud formation of the shoot should aid materially in checking the spread of the disease. The production of a smaller amount of growth which is subject to the rapid invasion of the organism would it seems give fewer cankers and less over-wintering.

Application of Present Investigations to Methods of Fire Blight Control

A considerable amount of work remains to be done on various phases of the fire blight problem. As has been pointed out, the matter of the over-wintering of the organism has not been investigated thoroughly enough. If the organism over-winters in small twigs in a fairly large percentage of the cases in any given year it will mean that much more careful work must be done by the average operator in removing blighted twigs and cankers from the orchard during the dormant season. Old, apparently dead cankers must not be left in the tree as they too, as has been shown, may serve to aid in over-wintering.

The evidence at hand seems to show that bees and other nectar seeking insects cause more damage through the spread of blossom infection from tree to tree than do the other insects which are reported to be of importance.

Meteoric water is no doubt much more effective, in spreading the organisms to both blossoms and twigs from the primary infections, than any of the insects. The nectaries of the flowers and the stomata of the young developing leaves are in a very receptive condition during rainy periods. As was shown by Gossard and Walton (12), within 72 hours after pollination the blossoms cease to be liable to infection. It is likewise true that young leaves reach a stage of development in which infections are not produced through the stomata. The leaves however are in a susceptible condition over a longer period, as a rule, during a normal season than are the blossoms. The long period of unfolding of new leaves which occurs in excessively rainy springs and summers, keeps the twigs in a continued susceptible condition.

The demonstration of the actual occurrence of stomatal infection may aid

in the interpretation of many cases of twig infection for which a satisfactory explanation has heretofore been lacking.

Field inoculations have corroborated the evidence obtained in the greenhouse concerning stomatal infections.

Twig infection in Michigan seems to result from stomatal infection of the leaf. This is perhaps due to the slow growing conditions which are brought about by the fairly low temperatures during the early part of the growing season. These tend to hold back the leaf development and so allow for the penetration of the zoogloeal mass into the petiole and shoot.

The relationship which exists between the age of leaf and ease of infection enables an observer to determine whether or not the growth produced by any given tree may be susceptible to infection.

The writer would call attention to the significance of those findings as to the origin of twig infections because the nature of this infection process may play a very decisive role in the development of new and effective fire blight control measures, by sanitation and possibly by spraying.

Summary

1. Fire blight is one of the most important diseases of apple, pear, and quince in Michigan.

2. Over-wintering of the causal organism usually occurs in the marginal region of the cankers. However in two cases the organism has been found to remain alive from fall to the following spring in dead tissue as far as twelve inches within the canker edge. The percentage of cankers in which this may occur has not been determined.

3. Dissemination of the causal organism may be brought about by a number of different species of insects. Bees no doubt are an important factor in establishing sources of infection in the blossoming tree. Rain is clearly effective in spreading the disease to other blossoms after these primary sources have been established. Attempts to duplicate twig infection by aphids as reported by other investigators have given negative results on all but three of 800 twigs. About 4,500 aphids were used in these inoculations.

4. Periods of twig infection may be correlated rather closely with rainfall periods.

5. Infection of healthy leaves does occur commonly. Examination of stained microtome sections of infected leaves shows that primary infection in the leaf occurs through the stomata. Experiments in the greenhouse and in the field have given 75 and 25 per cent of infection respectively under optimum conditions.

6. Fresh wounds of leaves, fruit, limbs, and trunk no doubt serve as a considerable source of infection during rainfall periods.

7. Stomatal infection usually results in twig blight if the infection occurs in very young leaves of growing twigs. Leaf spot results if the infected leaf is nearly mature at the time of inoculation.

8. The movement of the pathogene in the tissue seems to be one of mass action, the organisms being confined to the jelly-like matrix in which they are embedded. In no case in any of the preparations examined were bacteria observed to be in advance of this matrix.

9. The most satisfactory control measures for fire blight consist of, (1) sanitary measures in the elimination of blighted parts, (2) the use of cultural practices which will induce a moderate twig growth, and (3) the use

of resistant varieties as stocks. The significance of the writers experiments in interpreting the fire blight situation as it occurs in the orchard is outlined, and the bearing of these findings on possible control measures is given.

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